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Circulating MicroRNAs as Potential Biomarkers for the Diagnosis of Thyroid Carcinoma: A Systematic Review and Meta-analysis

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Abstract: Thyroid cancer (TC) ranks as the most prevalent malignant endocrine neoplasm worldwide, holding 9th place in terms of occurrence and ranking 24th for fatality among all diagnosed malignant tumors. Thus, discovering efficient biomarkers for timely identification and diagnosis of TC is pivotal. A meta-analysis was conducted to evaluate the diagnostic capability of circulating microRNAs (miRNAs) in detecting TC, registered under INPLASY202360048 on the INPLASY website. A systematic search of four databases (PubMed, Embase, Web of Science, and Cochrane Library) was performed to identify relevant articles published from inception until December 22, 2022. Stata 14.0 software was used to calculate the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic ratio (DOR), and area under the summary receiver operating characteristic (ROC) curve to assess the accuracy of miRNAs in the diagnosis of TC. Study heterogeneity was quantitatively evaluated using the Cochran-Q test and I2 statistic. Given the significant variability among studies, we opted for a random-effect model. Subgroup analysis and regression analysis were conducted in an effort to identify any possible factors contributing to heterogeneity. Our meta-analysis included 935 TC patients and 914 non-TC controls across 48 studies from 15 articles. The results showed that the summary sensitivity and specificity were 0.80 (95% confidence interval [CI]: 0.76, 0.84) and 0.81 (95% CI: 0.77, 0.85), respectively, the combined positive likelihood ratio was 4.27 (95%CI:3.43,5.33), the negative likelihood ratio was 0.24 (95%CI:0.20,0.30), and the diagnostic ratio was 17.55(95%CI:12.26,25.12). The combined AUC is 0.88 (95%CI: 0.84, 0.90). MiRNA profiling, regulation mode of miRNAs, and cut-off value settings emerged as primary heterogeneity sources. Based on our meta-analysis, circulating miRNAs show promise as a non-invasive diagnostic biomarker for TC.

1. Introduction

Thyroid cancer, an epithelial tumor arising from thyroid follicular cells or adjacent tissue [1], has experienced a dramatic increase in incidence for lesions <2.0 cm in diameter in the US, with over a four-fold rise between 1983 and 2011[2]. All thyroid cancers, except for medullary thyroid cancer (MTC), have their origins in the follicular cells of the thyroid gland. Differentiated thyroid cancer (DTC) represents 88% of all thyroid neoplasms, comprising papillary thyroid carcinomas (PTCs), follicular thyroid carcinomas (FTCs), and Hürthle cell carcinomas [3]. As the majority of TCs remain asymptomatic and hidden, early detection is crucial to prognosis and disease prevention [4]. Despite ultrasound and fine-needle aspiration cytology (FNAC) being standard diagnostic modalities for TCs, postoperative histopathological examination remains the gold-standard technique [5]. However, these methods are invasive and highly reliant on the operator's technical performance and experience, particularly in the follicular neoplasms (FNs) category [6]. Therefore, there is a requirement for reliable and non-invasive biomarkers to improve diagnostic and therapeutic accuracy.

MicroRNAs (miRNAs) are a kind of non-coding RNA, about 22 nucleotides in length that is transcribed from endogenous genes. They perform a crucial role in critical cellular functions such as development, cell differentiation, inflammation, proliferation, apoptosis, and tumorigenesis [7]. Previous studies suggest that miRNA expression levels in thyroid carcinoma tissue can be either up-regulated or down-regulated compared to normal tissue, affecting roughly 32% and 38% of miRNAs, respectively. Moreover, there are substantial disparities in miRNA expression profiles among different subtypes of thyroid carcinoma [8]. Exosomes, which are intracellular vesicles involved in intercellular transport and communication regulation, have assumed a critical position in the biomarker field [9]. As they are stable, non-invasive, resistant to ribonuclease degradation, and readily available, miRNAs separated from serum, plasma, or exosomes seem to be promising biomarkers for diagnosing TC.

The previous studies concluded that miRNAs have significant potential as a diagnostic tool for TC [10, 11]. Nevertheless, there are currently no circulating miRNAs available that are clinically feasible for the dependable diagnosis of TC. Consequently, we conducted this meta-analysis to assess the feasibility of miRNAs as a non-invasive diagnostic marker for TC. Our findings may facilitate early detection and intervention for individuals with thyroid cancer.

2. Materials and Methods

Registration of our protocol on INPLASY (INPLASY202360048), along with the corresponding details, is available at INPLASY.COM. This meta-analysis follows the PRISMA-DTA statement of preferred reporting items for systematic reviews and meta-analyses of diagnostic test accuracy [12].

2.1. Search Strategy

To guarantee the comprehensiveness of our analysis, we searched four databases (PubMed, Embase, Web of Science, and Cochrane Library) until December 22, 2022. The search strategy followed the Population, Intervention, Comparison, Outcome, and Study design (PICOS) principles, encompassing all relevant Medical Subject Headings (MeSH) and entry words obtained from the National Center for Biotechnology Information (NCBI) website. Additional File 1 provides detailed information on the complete search strategy and keywords utilized in the search. To further enhance the completeness of our investigation, we conducted a manual search of applicable articles to complement the search process.

2.2. Eligibility Criteria

To assure the accuracy and dependability of our study, we established strict inclusion criteria for subject recruitment. These criteria comprised (1) utilizing clinically recognized diagnostic criteria to diagnose all patients in the case group, (2) characterizing the intervention by diagnosing TMN through miRNA examination, and (3) directly obtaining or computing false positive (FP), true positive (TP), false negative (FN), and true negative (TN) from the literature. To maintain high-quality research standards, we excluded studies involving non-human trials, non-case-control designs, reviews, letters, or conference abstracts, and also eliminated studies lacking sufficient data from our analysis.

2.3. Data Extraction and quality assessment

Two researchers independently extracted relevant data and information from eligible studies, including first author, year of publication, country, miRNAs profiling, internal reference, cut-off values, comparison type, sample size, sample type, miRNAs detection method, AUC with 95% confidence intervals (CIs), and diagnostic performance data (sensitivity, specificity, TP, FP, TN, FN).

Following this, we assessed the quality of the studies using Rev Man 5.3 software and the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [13], which included four domains: patient selection, index test, reference standard, and flow and timing.

2.4. Statistical Analysis

We extracted sample size, sensitivity, and specificity from each study to calculate TP, FP, FN, and TN using Rev Man 5.3 software. The meta-analysis was performed in Stata 14.0 software, generating pooled estimates of sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and summary receiver operating characteristic (SROC) with 95% confidence intervals (CIs). The area under the curve (AUC) of the SROC was utilized to assess diagnostic efficacy, with values from 0.5-0.7, 0.7-0.9, and 0.9-1.0 indicating low, moderate, and high efficacy, respectively [14]. The threshold effect was examined using Spearman's correlation coefficient and P-value via Meta-DiSc 1.4 software [15]. Heterogeneity among studies was evaluated through Q-test and I^2 statistics, with I^2 values $> 50\%$ and P-values < 0.05 indicated significant heterogeneity [16], necessitating a random-effects model [17]. Heterogeneity sources were investigated through subgroup analyses and meta-regression. Publication bias was assessed utilizing Deek's funnel plot, with P-values <0.1 suggesting potential bias [18].

3. Results

3.1. Literature Search and Study Characteristics

PubMed, Embase, WoS, and Cochrane Library (2012-2021) were queried; 582 records were retrieved and 335 remained post-duplicate removal (n=247). Then 168 irrelevant articles were excluded. We conduct secondary eligibility assessment based on the abstract, and 108 studies were filtered out. Ultimately, 15 articles were included in this meta-analysis after screening 59 full-text articles. Figure 1 illustrates the screening flow, and Table 1 outlines the exclusion criteria.

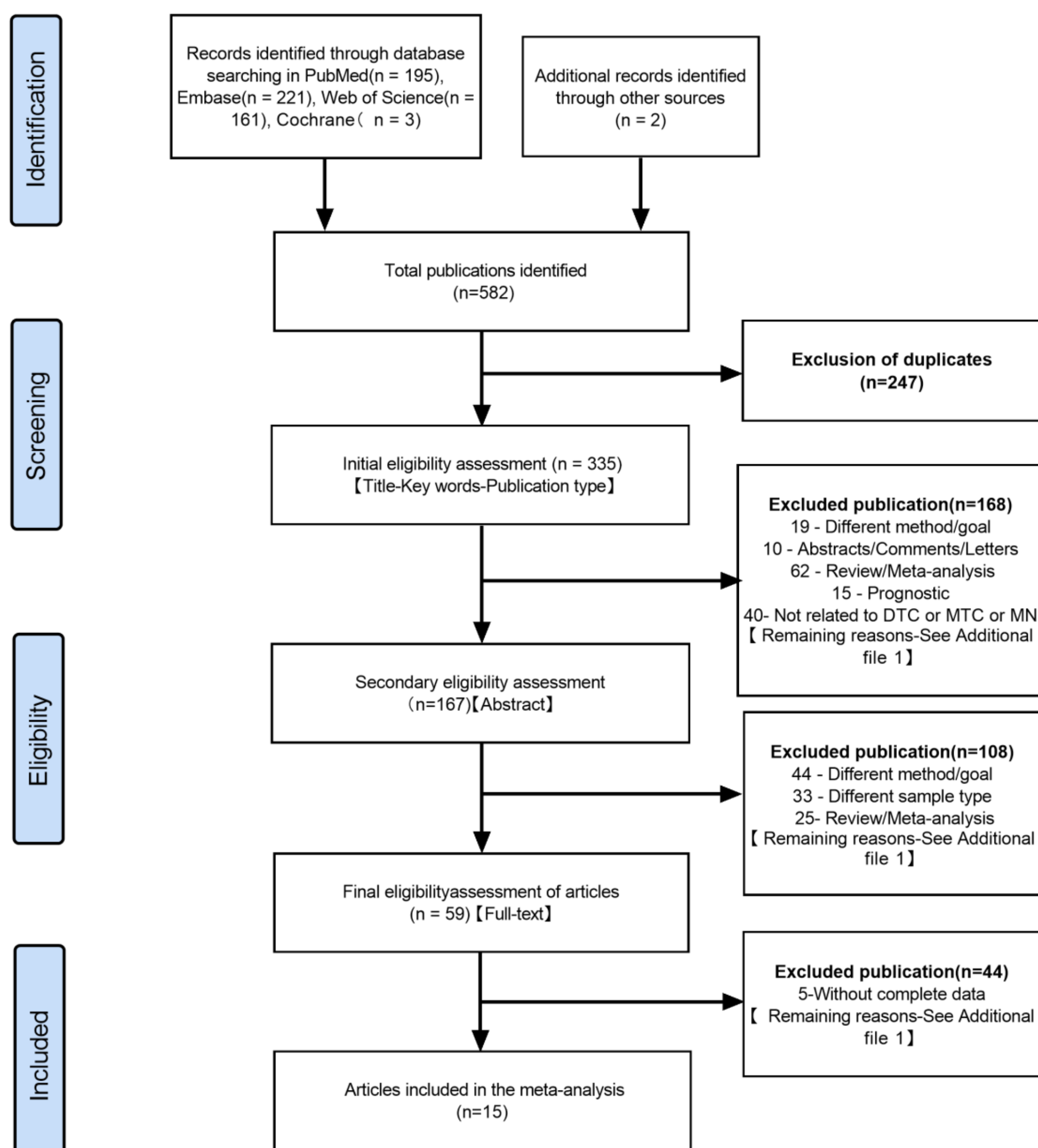

Figure 1: Flow diagram of screening process for eligible studies

Table 1: Summary of exclusion criteria for all stages of eligibility assessment

Reason for exclusion	**Number**
Different method/goal	86
Abstracts/Comments/Letters	10
Different sample type	45
Metastatic focus	10
Not related to DTC or MTC or MN	42
Prognostic	18
Review/Meta-analysis	90
Therapeutic	14
Without complete data	5
Total excluded publications	320

Our meta-analysis included 48 case-control studies from 15 articles (Table 2), involving 935 patients diagnosed with thyroid carcinoma and 914 controls. The control groups were sourced from healthy populations or individuals diagnosed with benign thyroid nodules (BTN), non-C-cell thyroid nodular (non-CTN), or pheochromocytoma (PCC).

Table 2: Study characteristics analyzed in the meta-analysis.

First Author /Year	Country	miRNAs	Expression	Reference	cut-off	Comparison type	case/Control	Method	Specimen	Sen(%)	Spe(%)	AUC(95%CI)
Yu,S 2012	China	let-7e	upregulated	miR-16	1.41	PTC / BTN	106 / 95	qRT-PCR	serum	63.20	89.50	0.782
Yu,S 2012	China	miR-151-5p	upregulated	miR-16	1.08	PTC / BTN	106 / 95	qRT-PCR	serum	59.40	89.50	0.780
Yu,S 2012	China	miR-222	upregulated	miR-16	1.39	PTC / BTN	106 / 95	qRT-PCR	serum	81.10	89.50	0.906
Yu,S 2012	China	let-7e miR-151-5p miR-222	upregulated	miR-16	0.41	PTC / BTN	106 / 95	qRT-PCR	serum	87.80	88.40	0.917
Yu,S 2012	China	let-7e	upregulated	miR-16	0.99	PTC / HC	106 / 44	qRT-PCR	serum	78.30	72.70	0.786
Yu,S 2012	China	miR-151-5p	upregulated	miR-16	0.68	PTC / HC	106 / 44	qRT-PCR	serum	79.20	68.20	0.776
Yu,S 2012	China	miR-222	upregulated	miR-16	0.8	PTC / HC	106 / 44	qRT-PCR	serum	94.30	70.50	0.882
Yu,S 2012	China	let-7e miR-151-5p miR-222	upregulated	miR-16	0.58	PTC / HC	106 / 44	qRT-PCR	serum	86.80	79.50	0.897
Cantara,S 2014	Italy	miRNA95	downregulated	ath-miR-159a miR-16 miR-451	0.0005	PTC / BTN	79 / 80	qRT-PCR	serum	94.90	98.70	NA
Cantara,S 2014	Italy	miRNA29b	downregulated	ath-miR-159a miR-16 miR-451	3.2043	PTC / BTN	79 / 80	qRT-PCR	serum	48.10	85.00	NA
Cantara,S 2014	Italy	miRNA579	downregulated	ath-miR-159a miR-16 miR-451	0.0005	PTC / BTN	79 / 80	qRT-PCR	serum	78.50	85.00	NA
Cantara,S 2014	Italy	miRNA190	upregulated	ath-miR-159a miR-16 miR-451	>0.0191	PTC / BTN	79 / 80	qRT-PCR	serum	93.70	78.70	NA
Li.M 2015	China	miR-25-3p	upregulated	U48 RNA	1.41	PTC / BTN	56 / 95	qRT-PCR	serum	92.80	68.80	0.835
Li.M 2015	China	miR-451a	upregulated	U48 RNA	1.38	PTC / BTN	56 / 95	qRT-PCR	serum	88.90	66.70	0.857
Li.M 2015	China	miR-25-3p miR-451a	upregulated	U48 RNA	1.25	PTC / BTN	56 / 95	qRT-PCR	serum	95.60	64.10	0.863
Lee,YS 2015[19]	Korea	miR-146b	upregulated	NA	NA	PTC / BTN	70 / 19	qRT-PCR	plasma	64.10	57.90	0.649
Lee,YS 2015	Korea	miR-155	upregulated	NA	NA	PTC / BTN	70 / 19	qRT-PCR	plasma	74.30	63.20	0.695
Yu,S 2016[20]	China	miR-124-3p	upregulated	NA	2.04	PTC / BTN+HC	50 / 100	qRT-PCR	serum	88.00	78.80	0.859
Yu,S 2016	China	miR-9-3p	upregulated	NA	1.7	PTC / BTN+HC	50 / 100	qRT-PCR	serum	80.00	73.70	0.823
Yu,S 2016	China	miR-124-3p	upregulated	NA	2.04	PTC / BTN	50/50	qRT-PCR	serum	88.00	76.00	0.831
Yu,S 2016	China	miR-9-3p	upregulated	NA	1.7	PTC / BTN	50/50	qRT-PCR	serum	70.00	64.00	0.753
Yu,S 2016	China	miR-196b-5p	downregulated	NA	1.545	PTC / BTN	50/50	qRT-PCR	serum	74.00	66.00	0.781
Zhang,YQ 2017	China	miR-222	upregulated	miR-16	1.21	PTC / BTN	106 / 35	qRT-PCR	serum	62.86	88.24	0.840
Zhang,YQ 2017	China	miR-221	upregulated	miR-16	2.51	PTC / BTN	106 / 35	qRT-PCR	serum	85.71	52.94	0.704
Zhang,YQ 2017	China	miR-146b	upregulated	miR-16	1.94	PTC / BTN	106 / 35	qRT-PCR	serum	94.29	68.24	0.873
Zhang,YQ 2017	China	miR-222 miR-221 miR-146b	upregulated	miR-16	0.7	PTC / BTN	106 / 35	qRT-PCR	serum	72.94	94.29	0.956
Zhang,YQ 2017	China	miR-222	upregulated	miR-16	1.46	PTC / HC	106 / 40	qRT-PCR	serum	74.12	90.00	0.876
Zhang,YQ 2017	China	miR-221	upregulated	miR-16	1.49	PTC / HC	106 / 40	qRT-PCR	serum	83.53	87.50	0.918
Zhang,YQ 2017	China	miR-146b	upregulated	miR-16	1.83	PTC / HC	106 / 40	qRT-PCR	serum	69.41	97.50	0.896
Zhang,YQ 2017	China	miR-222 miR-221 miR-146b	upregulated	miR-16	0.73	PTC / HC	106 / 40	qRT-PCR	serum	80.00	97.50	0.903
Zhang,MF 2017[21]	China	miR-451	downregulated	NA	2.96	PTC / BTN	70 / / 70	qRT-PCR	serum	40.00	85.70	0.626
Jahanbani,I 2018[22]	Kuwait	miR-222-3p	upregulated	miR-39	NA	PTC / BTN	81 / 32	qRT-PCR	serum	78.70	80.00	0.870
Zhang,YQ 2018	China	miR-222	upregulated	miR-16	1.59	PTC / BTN	58 / 35	qRT-PCR	serum	60.53	92.50	0.821
Zhang,YQ 2018	China	miR-221	upregulated	miR-16	2.37	PTC / BTN	58 / 35	qRT-PCR	serum	77.14	50.00	0.650
Zhang,YQ 2018	China	miR-146b	upregulated	miR-16	1.74	PTC / BTN	58 / 35	qRT-PCR	serum	77.14	78.95	0.900
Zhang,YQ 2018	China	miR-21	upregulated	miR-16	6.06	PTC / BTN	58 / 35	qRT-PCR	serum	88.57	89.47	0.950

Zhang,YQ 2018	China	miR-222、miR-221 miR-146b、miR-21	upregulated	miR-16	NA	PTC / BTN	58 / 35	qRT-PCR	serum	91.43	92.11	0.971
Xu,JH 2019[23]	China	miR-663	downregulated	U6	2.76	PTC / BTN	68 / 40	qRT-PCR	plasma	87.00	54.80	0.208
Yin,G 2020[24]	China	miR-130a-3p	downregulated	U6	NA	DTC/ BTN	40 / 40	qRT-PCR	exosomal	88.80	90.80	0.828 (0.763-0.881)
Mohamed, S.A. 2020[25]	Egypt	miRNA-222	upregulated	NA	> 0.62	MN / BTN	8 / 37	qRT-PCR	serum	50.00	32.43	NA
Liang,MH 2020[26]	China	miR-16-2-3p	upregulated	miR-30e-5p	NA	PTC / BTN	35 / 30	qRT-PCR	exosomal	68.57	66.67	0.690
Liang,MH 2020	China	miR-223-5p	upregulated	miR-30e-5p	NA	PTC / BTN	35 / 30	qRT-PCR	exosomal	57.14	80.00	0.680
Liang,MH 2020	China	miR-16-2-3p miR-223-5p	upregulated	miR-30e-5p	NA	PTC / BTN	35 / 30	qRT-PCR	exosomal	54.29	90.00	0.710
Liang,MH 2020	China	miR-16-2-3p miR-223-5p miR-34c-5p	upregulated	miR-30e-5p	NA	PTC / BTN	35 / 30	qRT-PCR	exosomal	60.00	86.67	0.720
Liang,MH 2020	China	miR-16-2-3p 、miR-223-5p miR-34c-5p、miR-101-3p	upregulated	miR-30e-5p	NA	PTC / BTN	35 / 30	qRT-PCR	exosomal	71.43	73.33	0.740
Liang,MH 2020	China	miR-223-5p、miR-34c-5p miR-101-3p、miR-146b-5p	upregulated	miR-30e-5p	NA	PTC / BTN	35 / 30	qRT-PCR	exosomal	74.29	66.67	0.730
Censi,S 2021[27]	Italy	miR-375	upregulated	hsa-miR-24-3p	>2.1	MTC /non-CTN +PCC + HC	68/82	qRT-PCR	serum	92.60	97.60	0.978
Li,SH 2021[28]	China	miR-48a-p	downregulated	U6	NA	DTC/ BTN	40 / 40	qRT-PCR	exosomal	88.80	90.80	0.828

Up, upregulated; down, downregulated; NA, not available; PTC, papillary thyroid carcinoma; BTN, benign thyroid nodules; HC, healthy controls; DTC, differentiated thyroid cancer; MTC, medullary thyroid carcinoma; non-CTN, non-C-cell thyroid nodular; PCC: pheochromocytoma

For miRNAs detection, all articles included in our analysis employed Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). Of these, among them, 37 studies detected miRNAs in serum, three in plasma, and eight in plasma exosomes. Of the 38 studies, single miRNAs were reported while ten discussed multiple miRNAs. The majority of studies focused on Asian populations, with only five conducted in non-Asian populations. Out of the 44 studies, 36 provided cut-off values for miRNA detection. Specifically, six studies examined miRNA-222, four analyzed miRNA-146b, and three included the examination of miRNA-221.

3.2. Quality Assessment

Quality assessment of the studies was conducted using Rev Man 5.3 software and the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. As all individuals diagnosed with TC were identified based on established clinical criteria, all of the included trials adopted case-control designs, which posed significant risks in the selection field. Additionally, due to the retrospective nature of these studies, a high risk of bias existed in the index test domain. The results of this assessment are presented in Figure 2.

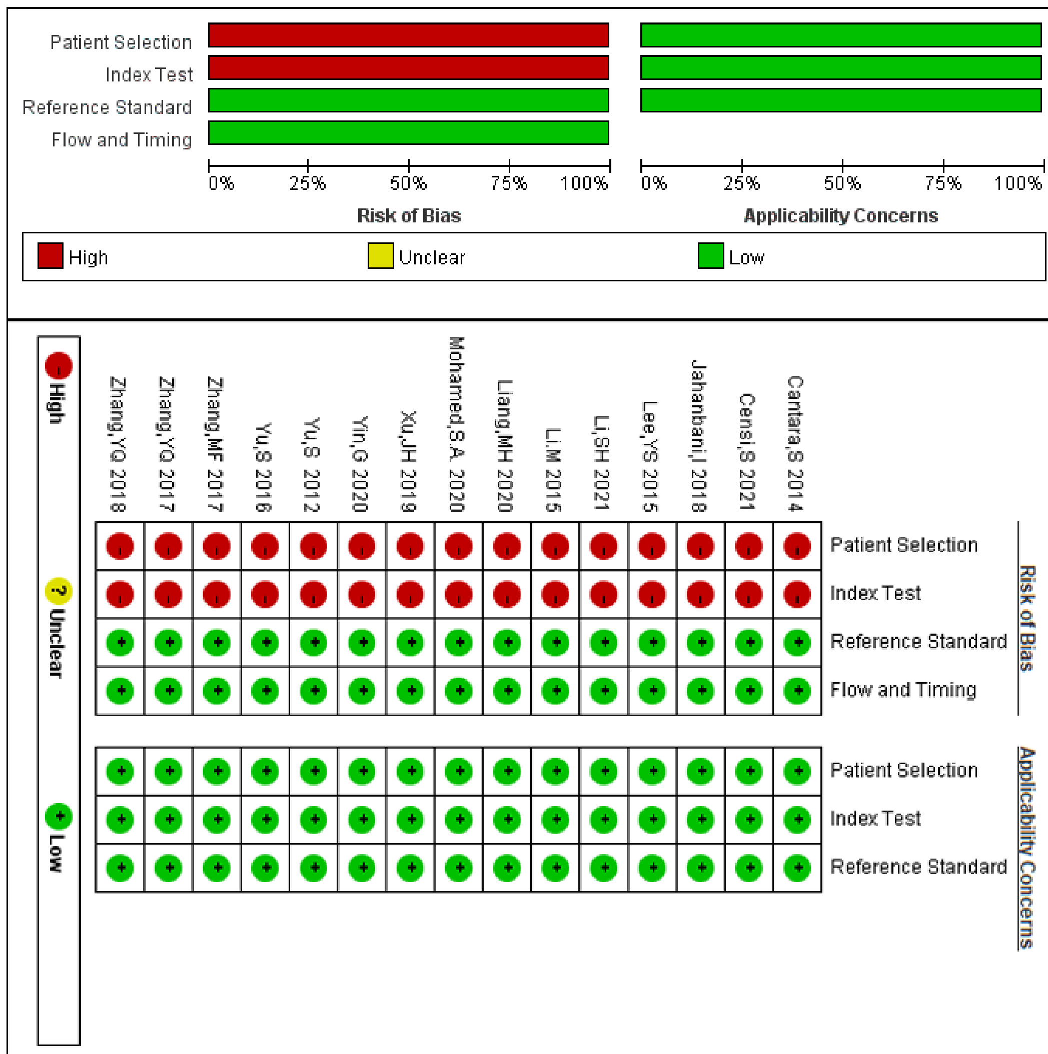

Figure 2: The diagnostic accuracy studies were evaluated for quality using the QUADAS-2 tool, with risk of bias and applicability indicated by red, yellow, and green colors.

3.3. Diagnostic Value of miRNAs

The forest plot (Figure 3) revealed high heterogeneity among studies, with I2 values exceeding 50% (86.72% for sensitivity and 82.67% for specificity). Consequently, a random-effects model was employed to evaluate the diagnostic accuracy of miRNAs in patients with PTC, DTC, MTC, or MN. The pooled results showed a sensitivity of 0.80 (95% CI, 0.76-0.84), specificity of 0.81 (95% CI, 0.77-0.85), PLR of 4.27 (95% CI, 3.43-5.33), NLR of 0.24 (95% CI, 0.20-0.30), and DOR of 17.55 (95% CI, 12.26-25.12). Additionally, the AUC was calculated as 0.88 (95% CI, 0.84-0.90) (Figure 4). We then explored the threshold effect using Meta-DiSc 1.4 software, which yielded a non-existent threshold effect with a Spearman's correlation coefficient of -0.020 and a P-value of 0.895. To further appraise the clinical utility of miRNAs, we calculated PLR and NLR, where values of PLR >10 and NLR <0.1 indicate high diagnostic accuracy. Notably, miR-222, miRNA190, miR-146b, miR-25-3p, and miR-451a emerged as potential miRNAs meriting further research (Figure 5A), based on studies by Yu et al.[29], Cantara et al.[18],Li et al.[30],Zhang et al.[31] Furthermore, we presented a Fagan's nomogram in Figure 5B, illustrating positive post-test and

negative post-test probabilities of 52% and 6%, respectively, when the prior probability was set to 20%. This suggests that individuals with dysregulated miRNAs have a 52% chance of being diagnosed with TC, while those with normal miRNA expression levels have a 6% chance of being diagnosed with TC.

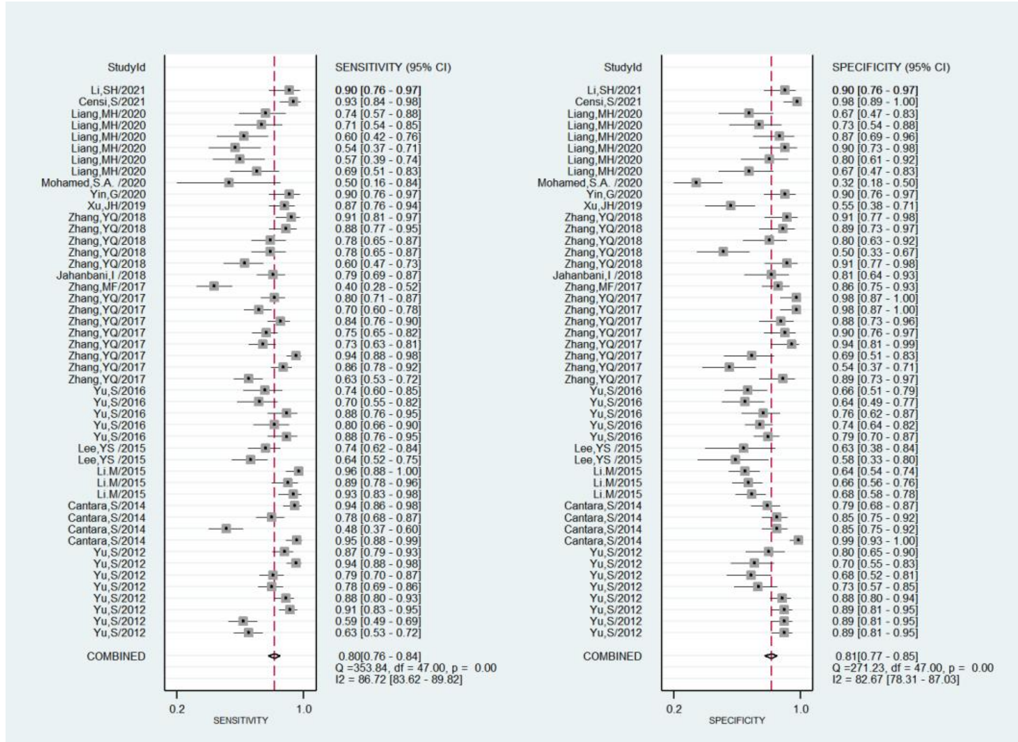

Figure 3: Forest plots demonstrating miRNA sensitivity and specificity in TC diagnosis, including corresponding confidence intervals on the right.

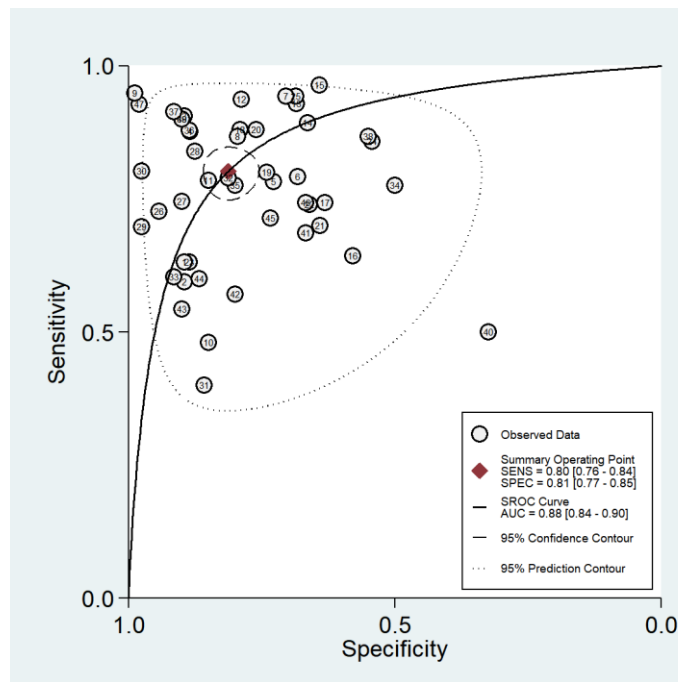

Figure 4: The summary receiver operating characteristic (sROC) curve provides an assessment of Overall miRNA accuracy in TC diagnosis.

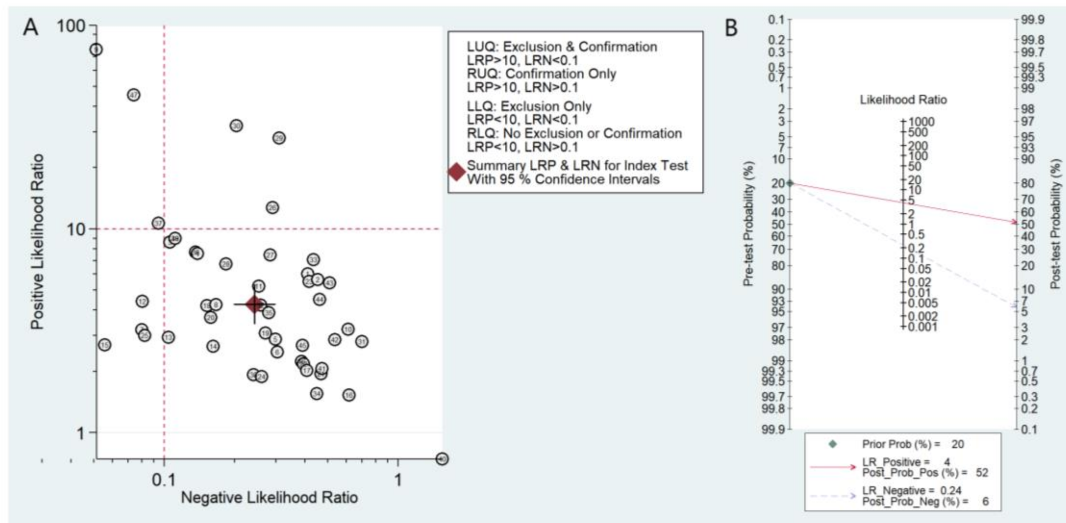

Figure 5: Assessment of miRNA clinical applicability for TC diagnosis (A) Summary of positive and negative likelihood ratios. (B) Fagan's nomogram evaluates clinical tility.

3.4. Subgroup Analyses, Meta-Regression

We investigated potential sources of heterogeneity by conducting subgroup analysis (Table 3) and regression analysis, categorizing the studies based on miRNA profiling, comparison type, sample size, miRNA expression, ethnicity and cut-off values setting. Our findings indicated that several factors significantly influenced the diagnostic performance, including multiple miRNA assays, downregulated miRNAs, sample sizes larger than 100, non-Asian populations, and cut-off values.

Table 3: Summary estimates of diagnostic power and their 95% confidence intervals.

Subgroup	**No. studies**	**Sen (95% CI)**	**Spe (95% CI)**	**PLR (95% CI)**	**NLR (95% CI)**	**DOR (95% CI)**	**AUC (95% CI)**	**I^2(%)**
MiRNA profiling								
Single miRNA	38	0.80(0.75-0.84)	0.80(0.75-0.84)	4.01(3.13-5.14)	0.25(0.20-0.31)	16.18(10.66-24.54)	0.87(0.84-0.90)	78.00
Multiple miRNAs	10	0.81(0.71-0.87)	0.85(0.77-0.91)	5.54(3.55-8.65)	0.23(0.15-0.34)	24.50(13.04-46.04)	0.90(0.87-0.92)	67.60
Comparison type								
PTC / BTN	34	0.79(0.73-0.83)	0.80(0.75-0.84)	3.92(3.13-4.90)	0.27(0.21-0.34)	14.71(10.01-21.61)	0.86(0.83-0.89)	75.90
PTC / HC	8	0.82(0.76-0.86)	0.86(0.75-0.92)	5.67(3.25-9.90)	0.21(0.17-0.27)	26.57(14.59-48.37)	0.89(0.86-0.92)	61.40
Sample size								
<100	16	0.74(0.67-0.80)	0.78(0.69-0.85)	3.37(2.22-5.11)	0.33(0.24-0.46)	10.13(5.07-20.23)	0.82(0.79-0.85)	77.40
≥100	32	0.83(0.78-0.87)	0.82(0.78-0.86)	4.71(3.67-6.05)	0.21(0.16-0.27)	22.40(15.19-33.03)	0.89(0.86-0.92)	74.80
Regulation mode								
Down-regulate	8	0.80(0.64-0.90)	0.86(0.74-0.93)	5.63(2.78-11.42)	0.24(0.12-0.46)	23.76(6.80-83.09)	0.90(0.87-0.92)	85.70
Up-regulate	40	0.81(0.76-0.85)	0.78(0.73-0.82)	3.63(2.90-4.55)	0.25(0.20-0.32)	14.54(9.64-21.94)	0.86(0.83-0.89)	76.50
Ethnicity								
Non-Asian	5	0.87(0.69-0.95)	0.92(0.80-0.97)	10.91(3.78-31.44)	0.14(0.06-0.38)	75.48(12.27-464.25)	0.96(0.93-0.97)	90.20
Asian	43	0.79(0.75-0.83)	0.80(0.75-0.83)	3.88(3.16-4.76)	0.26(0.21-0.31)	15.04(10.89-20.76)	0.86(0.83-0.89)	73.70
Cut-off values								
Given	36	0.82(0.77-0.86)	0.82(0.76-0.86)	4.44(3.40-5.80)	0.22(0.18-0.28)	19.91(13.17-30.10)	0.89(0.86-0.91)	77.70
NA	12	0.75(0.67-0.82)	0.80(0.73-0.86)	3.77(2.60-5.48)	0.31(0.22-0.44)	12.06(6.17-23.57)	0.85(0.81-0.88)	72.8

The diagnostic precision of miRNA panels was superior to individual miRNAs. The sensitivity, specificity, PLR, NLR, DOR and AUC values for single miRNAs and miRNA panels were 0.80 (95% CI: 0.75-0.84), 0.80 (95% CI:0.75-0.84), 4.01 (95% CI: 3.13-5.14), 0.25 (95% CI:0.20-0.31), 16.18(95% CI: 10.66-24.54) and 0.87 (95% CI: 0.84-0.90) and 0.81 (95% CI:0.71-0.87), 0.85 (95% CI: 0.77-0.91),5.54(95% CI: 3.55-8.65), 0.23(95% CI: 0.15-0.34), 24.50 (95% CI: 13.04-46.04) and 0.90 (95% CI: 087-0.92), respectively. Additionally, miRNA expression levels were associated with

diagnostic value. Downregulated miRNAs demonstrated better diagnostic performance, with 0.80 (95% CI: 0.64-0.90), 0.86(95% CI: 0.74-0.93), 5.63 (95% CI: 2.78-11.42), 0.24 (95% CI: 0.12-0.46), 23.76 (95% CI: 6.80-83.09) and 0.90 (95% CI: 0.87-0.92). Conversely, upregulated miRNAs had lower values, with sensitivity, specificity, PLR, NLR, DOR, and AUC values of 0.81 (95% CI: 0.76-0.85), 0.78(95% CI: 0.73-0.82), 3.63 (95% CI: 2.90-4.55), 0.25 (95% CI: 0.20-0.32), 14.54 (95% CI: 9.64-21.94) and 0.86 (95% CI: 0.83-0.89). Moreover, studies with sample size ≥ 100 exhibited significantly better diagnostic accuracy than those with sample size < 100, with sensitivity of 0.83 (95% CI: 0.78-0.87) vs. 0.74(95% CI: 0.67-0.80), specificity of 0.82 (95% CI: 0.78-0.86) vs. 0.78 (95% CI:0.69-0.85), and AUC of 0.89 (95% CI: 0.86-0.92) vs. 0.82 (95% CI: 0.79-0.85). The diagnostic value of miRNAs differed by ethnicity, with non-Asian populations outperforming Asian populations. However, the number of studies involving non-Asian populations was limited, so this finding may be coincidental. Moreover, studies that did not have cut-off values yielded pooled results for sensitivity, specificity, and AUC of 0.75 (95% CI: 0.67-0.82), 0.80 (95% CI: 0.73-0.86), and 0.85 (95% CI: 0.81-0.88), respectively. In contrast, studies with optimal cut-off values showed better diagnostic value with sensitivity of 0.82 (95% CI: 0.77-0.86), specificity of 0.82 (95% CI: 0.76-0.86), and AUC of 0.89 (95% CI: 0.86-0.91). In addition, PTC/HC had higher diagnostic accuracy of TC than PTC/BTN.

We conducted a meta-regression analysis (Figure 6) to pinpoint potential sources of heterogeneity in sensitivity and specificity. Our findings suggest that miRNA profiling, comparison type, sample size, miRNA expression levels, and cut-off value selection might account for the observed heterogeneity, while other covariates demonstrated no significant influence.

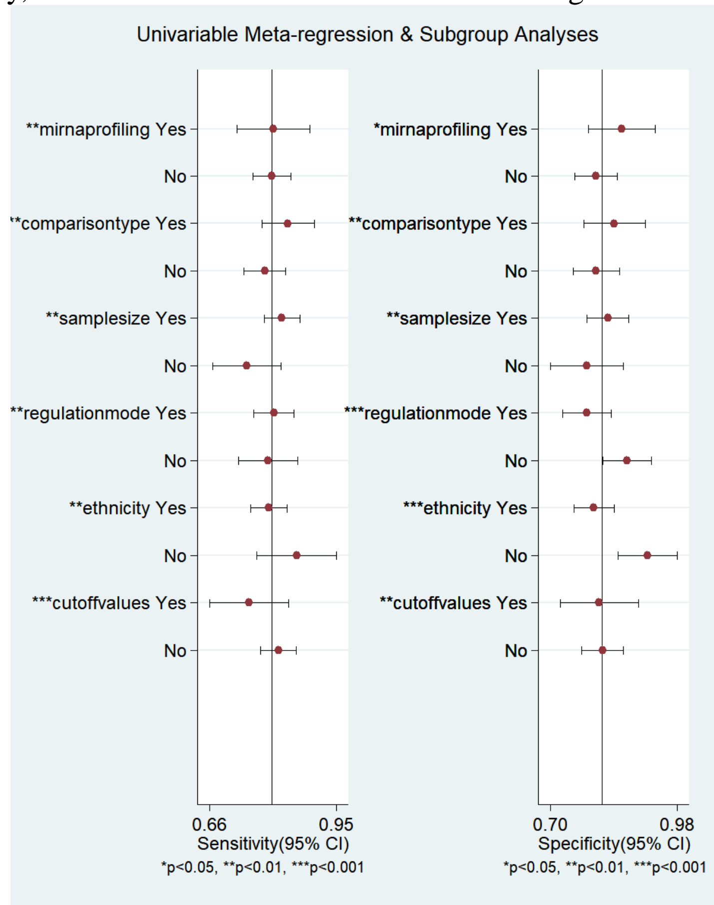

Figure 6: Univariable meta-regression and subgroup analyses to explore the main sources of heterogeneity.

3.5. Sensitivity Analyses

As shown in Figure 7, the sensitivity analysis confirmed that the random effects model was appropriate based on both the goodness of fit (Figure 7A) and bivariate normality (Figure 7B). Influence analysis revealed that Cantara et al[18], Li et al[30] , Zhang et al[32], Mohamed et al[25],and Censi et al[27] were the studies with the most significant impact on weight (Figure 7C).

Furthermore, outlier detection suggested that data from the studies conducted by Cantara et al, Zhang et al, and Mohamed et al. may have attributed to the heterogeneity (Figure 7D). May have contributed to the observed heterogeneity (Figure 7D). Upon the exclusion of these outlier studies, heterogeneity, as measured by I2, decreased to 2.20% for sensitivity and 4.50% for specificity. However, the combined findings related to diagnostic effectiveness did not significantly change (Table 4).

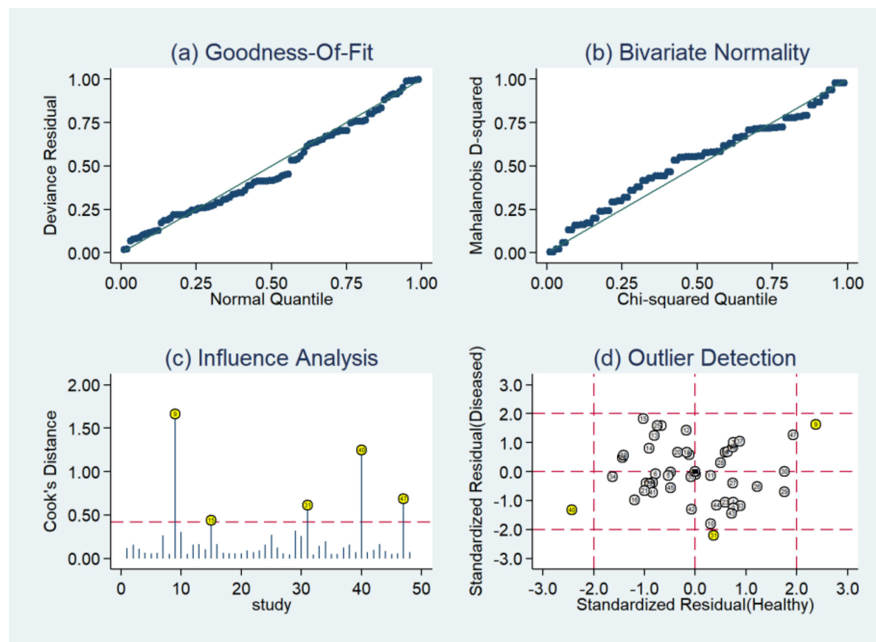

Figure 7: Figure of sensitivity analysis showing (A) goodness-of-fit; (B) bivariate normality; (C) influence analysis; (D) outlier detection.

Table 4: Diagnostic performance of miRNAs in thyroid carcinoma

Analysis	Overall	Outliers excluded
No. of studies	48	45
Sen (95% CI)	0.80(0.76-0.84)	0.81(0.77-0.84)
Spe (95% CI)	0.81(0.77-0.85)	0.81(0.77-0.84)
PLR (95% CI)	4.27(3.43-5.33)	4.22(3.49-5.12)
NLR (95% CI)	0.24(0.20-0.30)	0.24(0.20-0.29)
DOR (95% CI)	17.55(12.26-25.12)	17.64(13.01-23.92)
AUC (95% CI)	0.88(0.84-0.90)	0.88(0.85-0.90)

3.6. Publication Bias

Publication bias was evaluated employing Deek's funnel plot (Figure 8) and found an asymmetrical distribution (P=0.02), suggesting potential bias in the analyzed data.

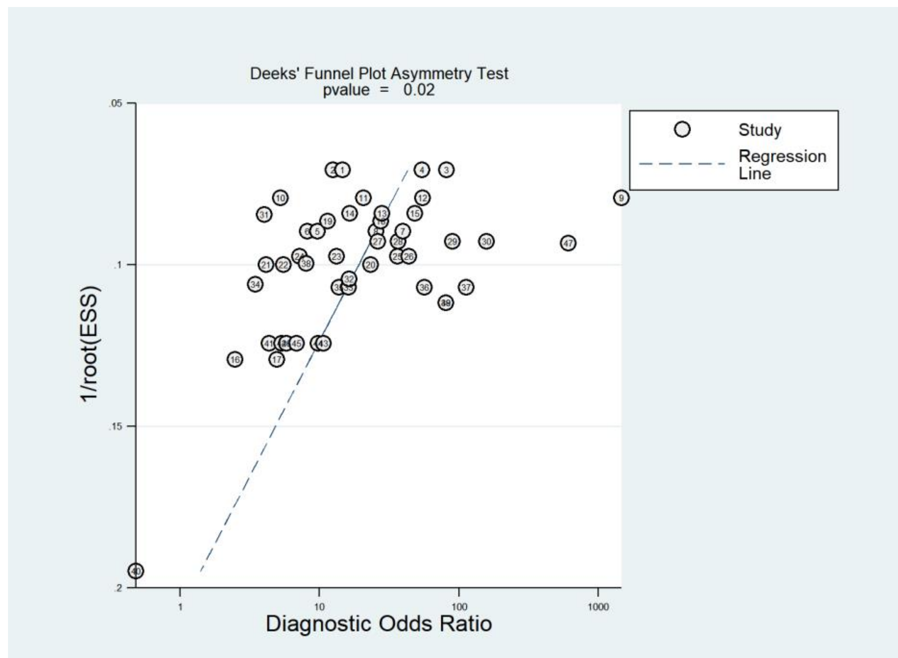

Figure 8: Deek's funnel plot to evaluate publication bias.

4. Discussion

Our meta-analysis included 15 eligible documents that encompassed 48 studies, which involved a total of 935 thyroid carcinoma patients and 914 controls. Based on the pooled results, miRNAs were found to have an estimated AUC of 0.88 (95% CI: 0.84-0.90), suggesting their potential utility in detecting TC. Notably, the sensitivity and specificity of miRNAs were high, with values of 0.80 (95% CI: 0.76-0.84) and 0.81 (95% CI: 0.77-0.85), respectively, for diagnosing TC. Additionally, our analysis yielded a pooled DOR value of 17.55, which suggests a relatively high level of overall accuracy in distinguishing between thyroid carcinoma patients and controls. The PLR value of 4.27 indicated that the probability of an individual with a positive test result having TC was approximately four times higher compared to those without TC. Conversely, the NLR value of 0.24 indicates that the likelihood of a patient having TC is around 24% if a negative result is obtained from the miRNAs assay.

The presence of heterogeneity in a meta-analysis can compromise the validity of a systematic review. We examined the threshold effect using Spearman correlation analysis ($r = -0.020$, $p = 0.895$), thus refuting the threshold effect. Subgroup and meta-regression analyses were performed to assess potential heterogeneity sources, considering miRNA profiling, comparison type, sample size, miRNA expression, ethnicity, and cut-off values. Our findings suggested that multiple miRNAs demonstrated greater accuracy than single miRNAs in diagnosing TC based on various parameters such as sensitivity (0.81 vs. 0.80), specificity (0.85 vs. 0.80), and AUC (0.90 vs. 0.87). This result is consistent with previous studies [10, 11]. However, there is currently no consensus on which specific miRNA panel should be used for TC diagnosis. Yu et al. [29] reported that let-7e, miR-151-5p, and miR-222 combined achieved SEN 0.87 and SPE 0.80. Zhang et al. [32] found miR-222, miR-221, miR-146b, and miR-21 resulted in SEN 0.91 and SPE 0.92. Similar meta-analyses reveal that multiple miRNAs with intricate molecular mechanisms can indicate tumor development, forming stable diagnostic networks and representing future trends.

Our subgroup analysis demonstrated that studies with sample sizes ≥100 had significantly higher diagnostic accuracy than those with sample sizes <100. These findings highlight the importance of

using larger research samples in future studies to improve the accuracy of TC diagnosis.

Other subgroup analysis revealed increased diagnostic accuracy in non-Asian populations compared to Asian populations, likely due to variations in disease prevalence, race-specific sensitivities, and fewer studies conducted on non-Asian populations. Additionally, we found that miRNAs that were downregulated had better diagnostic value than those that were upregulated. Specifically, we observed higher sensitivity (0.80 vs. 0.81), specificity (0.86 vs. 0.78), and AUC (0.90 vs. 0.86) for downregulated miRNAs, including miRNA95, miRNA29b, miRNA579, miR-196b-5p, miR-451, miR-663, miR-130a-3p, and miR-48a-p. Previous research has shown that a lack of expression of miRNA-29b promotes tumor metastasis and tumor epithelial mesenchymal transition [19]. Moreover, miR-451 downregulates in various tumor tissues, whereas its upregulation could increase the apoptosis rate of tumor cells [20]. Additionally, studies indicate a strong correlation between miR-663 expression and the invasion and migration of PTC cells [33].These miRNAs hold potential as biomarkers for TC diagnosis and therapeutic response evaluation. Furthermore, we found that studies with optimal cut-off values exhibited superior diagnostic value over those without cut-off values. Cut-off values play a crucial role in disease diagnosis. However, nearly a quarter of the included studies in our meta-analyses neglected to report cut-off values, potentially heightening bias risk in patient selection domain quality assessment. Consequently, future research should prioritize reporting cut-off values to ensure diagnostic accuracy.

Our meta-analysis aligns with conclusions from two prior studies by Xu et al.[10], and Chen et al.[11] However, our analysis offers several advantages: a larger sample size (15 articles with 48 studies), a more comprehensive list of miRNAs for improved diagnostic value assessment accuracy, and additional subgroup analyses to explore potential sources of heterogeneity. Thus, our study provides a robust and reliable evaluation of miRNAs' diagnostic value in TC.

Despite these strengths, our meta-analysis has limitations. Variability in laboratory and experimental methodologies could contribute to inconsistent findings. The predominance of Asian patient studies may introduce ethnic bias. Unaccounted patient factors like gender, age, and TNM stage might affect the reliability of our results. Additionally, the use of different internal reference genes across studies weakens our conclusions.

5. Conclusion

In conclusion, circulating miRNAs show promise as non-invasive diagnostic biomarkers for TC. To confirm our findings and optimize circulating miRNAs' efficacy for early detection, further high-quality case-control studies with multi-center and rigorous designs are required.

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